Bioavailability of pollutants and chemotaxis

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Abstract

The exposure of bacteria to pollutants induces frequently chemoattraction or chemorepellent reactions. Recent research suggests that the capacity to degrade a toxic compound has co-evolved in some bacteria with the capacity to chemotactically react to it. There is an increasing amount of data which show that chemoattraction to biodegradable pollutants increases their bioavailability which translates into an enhancement of the biodegradation rate. Pollutant chemoreceptors so far identified are encoded on degradation or resistance plasmids. Genetic engineering of bacteria, such as the transfer of chemoreceptor genes, offers thus the possibility to optimize biodegradation processes.

Introduction

Biodegradation of anthropogenic organic chemicals (AOCs) in natural and engineered environments is often not as efficient as expected due to a limited bioavailability, which represents the accessibility of a chemical for biotransformation and toxicity. As a result of sorption to soils and sediments, pollutants often only exhibit weak chemical activity gradients that promote their uptake and transformation by cells. Thus, the biodegradation rates may reflect the dependencies of restricted phase exchanges, and the pollutants, together with their environmental risks, may persist for longer periods of time (Figure 1). Research over the last decade has shown that the chemotactic movement of bacteria increases bioavailability, which in turn was found to have a beneficial role in bioremediation. In this review, we consider chemotaxis as the diverse tactic reactions to pollutants of bacteria exhibiting flagellar motility. The focus will be on AOCs, but due to their environmental relevance, the recent advances in the field of chemotaxis to inorganic pollutants, such as metals and nanomaterials, will also be reviewed.

Chemotaxis towards and away from pollutants

Chemotaxis has been extensively studied in enterobacteria that show chemotaxis to a limited number of compounds like amino acids, organic acids and sugars [1]. Many free-living bacteria have an increased number of chemoreceptors, which were shown to mediate chemotaxis to a wider range of compounds as compared to enterobacteria [2,3]. Interestingly, many of these compounds are chemicals of environmental concern. Chemoattraction was observed for example towards biphenyl, benzoic acid and chlorobenzoic acids [4**], toluene and its derivatives [5,6*], naphthalene and its derivatives [6*,7], nitroaromatics [8], chloroaromatics [9], chloronitroaromatics [10*],

aminoaromatics [11], explosives [12], aliphatic hydrocarbons [13] and herbicides [14*] in species like *Rhizobium* sp., *Bradyrhizobium* sp., *Pseudomonas* sp., *Azospirillum* sp., *Ralstonia* sp., *Burkholderia* sp. or *Flavimonasoryzihabitans*. In a significant number of cases the physiological relevance of chemoattraction to pollutants lies in the fact that these compounds serve as carbon and energy sources. This may be exemplified by the chemotaxis towards toluene and naphthalene by *Pseudomonas putida* DOT-T1E and G7, which possess specific degradation routes for both compounds, respectively [15,16]. In some cases chemoattraction was observed towards pollutants that are not metabolized by the bacterium and the physiological relevance of this behavior is little understood.

Given the toxic potential of most pollutants, it is conceivable that bacteria have also evolved chemorepellent responses. Bacterial repellence has been reported, for example, to hydrogen peroxide, hypochlorite and N-chlorotaurine [17], the PAHs anthracene and pyrene [18], Co^{++} and Ni^{++} [19], and silver nanoparticles [20]. Some chemicals can even be chemo-attractants for one bacterial species and be repellent for another [5,21,22]. The physical state of the chemical also appears to influence the type of response, since it was shown that the naphthalene degrader *P. putida* G7 was repelled by naphthalene in the vapor phase, whereas it was attracted when the compound was dissolved in the aqueous phase [23**]. In the light of such results one has to keep in mind that an observed chemotaxis phenotype can be the result of the action of several, potential antagonistic chemoreceptors that differ in their sensitivity to a given compound.

The complexity of the chemotactic reactions to pollutants is also illustrated by the dissimilar reactions exhibited by *P. putida* to silver nanoparticles and Ag^{+} ions [20].

Nano-scale silver induced a repellent response, possibly due to a direct effect of the nanoparticles on bacterial cells, and not due to the release of soluble Ag^{+} from the particles. Indeed, the bacterium did not show any repellent response to soluble Ag^{+}, what is in agreement with earlier reports, which demonstrate that some harmful chemicals, such as Cu^{++}, do not induce negative taxis in bacteria [19]. Furthermore a positive tactic response was detected at low concentrations of silver nitrate, what probably reflects the physiological role of Ag^{+} ions and other metal ions like Mn^{3+} and Fe^{3+} towards which taxis was observed [24-26]. The bacterial repellent responses can be considered as a prelude of toxicity because it is often observed only at sub-lethal pollutant concentrations, which suggests that taxis may indeed be part of survival strategies aimed on minimizing the deleterious effects of toxic compounds. In addition, the molecular machinery for the detection of chemicals for tactic purposes can also be employed for analytical purposes, namely the development of alternative bioassay methods.

Pollutant chemoreceptors so far identified are present on plasmids

The specificity of a chemotactic response is determined by chemoreceptors. Two chemoreceptors for aromatic pollutants have so far been described, which are NahY [27] of the naphthalene degrading *P. putida* G7 and McpT of the toluene, benzene and ethylbenzene degrading *P. putida* DOT-T1E [6*]. Both receptors mediate chemoattraction towards their respective degradation substrates. Interesting parallels exist between both receptors. NahY and McpT are encoded on plasmids pNAH7 [28] and pGRT1 [29], respectively. Both plasmids contain genes that are related to either degradation of or resistance to aromatic pollutants. The pNAH7 plasmid contains genes that encode the naphthalene degradation route and the *nahY* gene is co-transcribed with

part of these genes [27]. Plasmid pGRT1 contains two *mcpT* alleles which are both in vicinity of the *ttgGHI* operon that encodes the primary efflux pump responsible for solvent resistance [30]. McpT was found to mediate an extreme form of chemoattraction, termed hyperchemotaxis, towards a wide range of mono-and biaromatic compounds [6*]. The capacity of McpT to mediate a hyperchemotaxis response towards crude oil samples is illustrated in Figure 2. Due to taxis and a very high solvent resistance, cells were able to assemble on the surface of this toxic mixture of compounds. The deletion of the *mcpT* gene (Figure 2) abolished this capacity.

Although NahY and McpT exert a similar function, the sequence alignment of their ligand binding regions (LBRs) reveals no significant identity. However, close homologues of both receptors are found on other degradation plasmids. For example receptors showing 99 % sequence identity with McpT are found on the carbazole degradation plasmids pCAR1 of *P. resinovorans* CA10 [31] or the toluene degradation plasmid pWW53 of *P. putida* MT53 [32]. In analogy, NahY homologues are found on the naphthalene degradation plasmids pDTG1 [33] or pND6-1 [34]**.** This suggests that there are at least two different families of pollutant chemoreceptors, of which NahY and McpT are representative members.

A chemosensory signaling cascade is formed by chemoreceptors and cytosolic signaling proteins [1*]. Plasmids mentioned contain chemoreceptor genes but lack those of signaling proteins. This implies that chemotaxis is mediated by the concerted action of plasmid encoded receptors and genome encoded signaling proteins. A transfer of the *mcpT* gene into strains *P. putida* KT2440 and F1 conferred the hyperchemotaxis phenotype to both strains [6*]. This indicates that McpT is able to interact with the

signaling proteins, which offers the possibility of conferring pollutant chemotaxis to other bacteria by chemoreceptor gene transfer. Although these pollutant receptors are plasmid-encoded there is also evidence for genome-encoded pollutant chemoreceptors, since for example the plasmid-free strains *P. putida* KT2440 or F1 show chemotaxis towards toluene which, however, is in its magnitude inferior to the McpT-mediated hyperchemotaxis [6*].

Chemoattraction increases bioavailability of pollutants and enhances biodegradation rate

There is now sufficient evidence demonstrating that chemoattraction increases the bioavailability of pollutants. The best studied example is the capacity of *P. putida* G7 to degrade naphthalene. Grimm and Harwood [27] have proposed that NahY-mediated taxis towards naphthalene might facilitate its biodegradation. Proof of this hypothesis was brought by Aitken and co-workers. Using a heterogeneous aqueous system they were able to demonstrate that chemotaxis enhances naphthalene biodegradation [35]. Subsequent studies using chemotactic and non-chemotactic strains of *P. putida* G7 clearly demonstrated that chemotaxis increased naphthalene degradation when the compound is present in a non-aqueous-phase liquid [36]. Bioavailability can also be promoted by the chemotactic transport of *P. putida* G7 through fungal mycelia that act as pathways for mobilization [37*]. There are several studies that compare the capacity of microorganisms to degrade different pollutants with their capacity to chemotactically approach these compounds. Interestingly, in some cases chemotaxis was only observed towards compounds which were degraded by the microorganism whereas structurally similar non-substrate compounds were not found to be chemoattractants [10*,38], which confirms the link between chemotaxis and biodegradation.

The standard chemotaxis assays involve the measurement of motility in buffer solutions. However, pollutants adsorb frequently onto solids or into pores. To evaluate whether efficient pollutant chemotaxis occurs also under these more *in situ* conditions, chemotaxis assays were developed to monitor taxis in contaminated soils. Research based on close to *in situ* conditions has shown that pollutant chemotaxis occurs also in contaminated soil [39**] and in a reconstituted bench-scale microcosm [40**]. Biodegradation studies of carbon tetrachloride [41] and pesticides [42] have also demonstrated the ability of chemotaxis to enhance biodegradation under laboratory-conditions. It has also been shown in later research that bacterial motility and transport can be controlled through a suitable choice of chemical effectors [20,43,44*]. In well-controlled column systems, the influence of different effectors on the deposition of *P. putida* G7 was assessed in selected porous environments. Cellular deposition, however, was concomitantly dependent on the cellular motility (hyper-motility, attraction or repulsion), the sorption of the effector to the column packing material, and the resulting pore-water concentration (Figure 3).

Conclusions: Genetic engineering to improve resistance, degradation pathway expression and chemotactic mobilization.

The use of genetically engineered microorganisms for biodegradation purposes has been crowned with little success in the past. However, the reasons for the reduced efficiency were clearly identified and include, amongst others, limited bacterial resistance towards toxic compounds, inadequate expression of degradation pathways and bioavailability restrictions [45]. Research over the last decade has advanced enormously the understanding of the basic mechanisms related to these issues. The new knowledge on the functional link between chemotaxis and biodegradation will have implications for

the design of new bioaugmentation and biostimulation strategies oriented toward improving the performance of pollutant-degrading microbial populations. These strategies may overcome, for example, the limitations usually caused by the use of inoculants designed exclusively on the basis of their catabolic potential. In parallel, major conceptual and technical advances have been made in the field of synthetic biology, which now permit a much more rational and directed modification of the bacterial genome [46]. In the case of chemotaxis to pollutants it may be possible to engineer the process by transferring the chemoreceptor gene which establishes a signaling cascade with host signaling genes. Genetic engineering combining the acquired knowledge of pollutant related chemical processes with powerful synthetic biology approaches should give rise to biodegrading microorganisms that perform better than their predecessors.

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Figure Legends

Figure 1) Conceptual framework of this review. Biodegradation, bioavailability and chemotaxis are interconnected through a variety of concepts related to bacterial physiology and genetics, bioremediation performance and environmental risk.

Figure 2) Chemotaxis of *P. putida* DOT-T1E (A) and its mutant devoid of *mcpT* genes (B) towards undiluted crude oil recovered from the Spanish coast following the "Prestige" oil tanker accident. In the wild type strain cells accumulate right on the surface of this highly toxic mixture of hydrocarbons. Mutation of the *mcpT* gene abolished taxis. Reproduced with permission from [6*].

Figure 3) Effect of exposure of *Pseudomonas putida* G7 to salicylate (promoting positive taxis) and to silver nanoparticles (repellence) on bacterial transport through sand (A) and motile behavior (B-D). Salicylate significantly reduced deposition of G7 cells, whereas AgNPs enhanced attachment and caused filter blocking that resulted in a progressive decrease in deposition (A). Computer-aided analysis of cell trajectories showed (C-D) that exposure to salicylate induced smooth cell movement with no turning events (peaks in the rate of change of direction or RCDI), whereas cells exposed to AgNPs exhibited tortuous movement. Modified with permission from [44*].

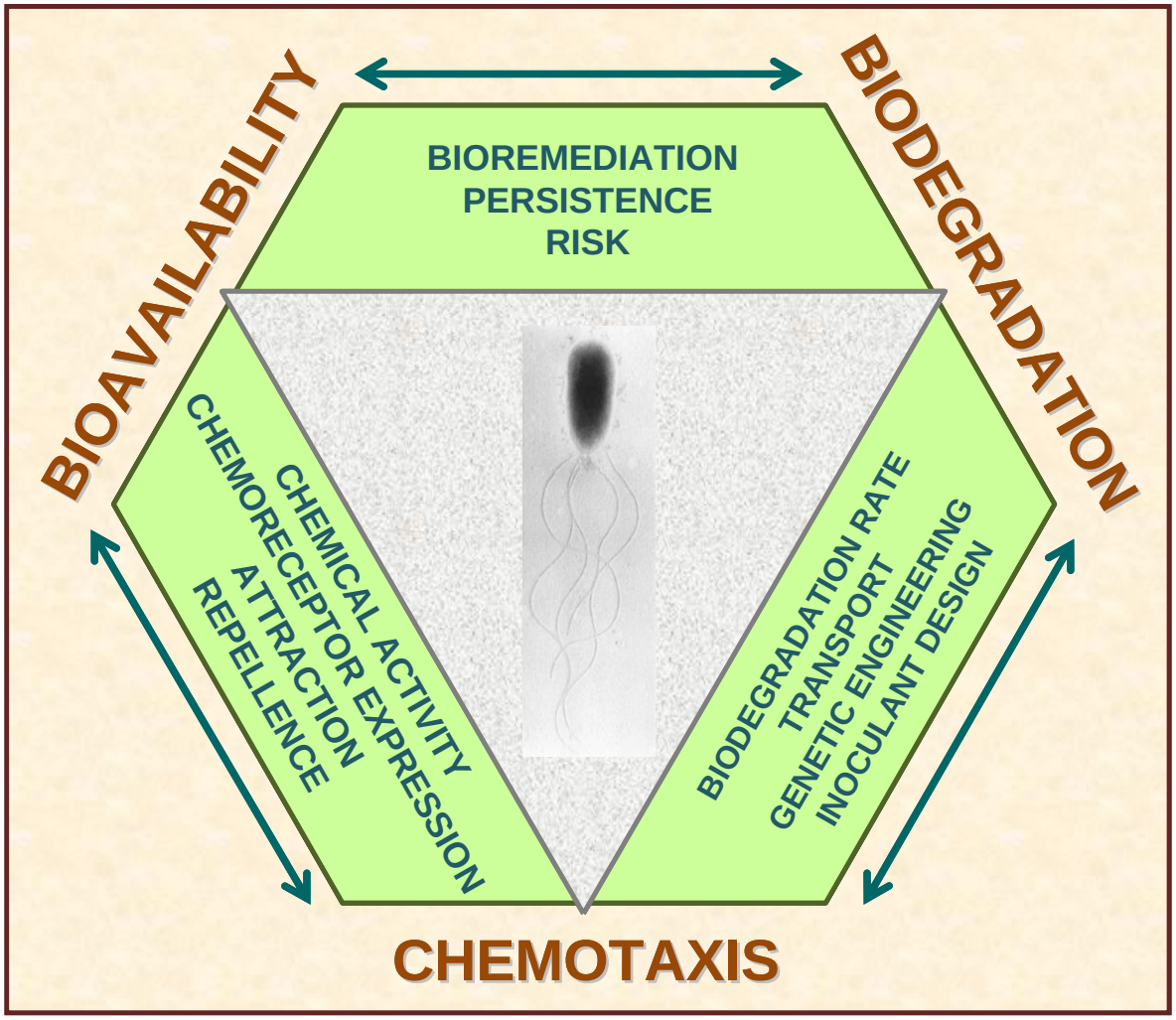

FIGURE 1. Krell et al.

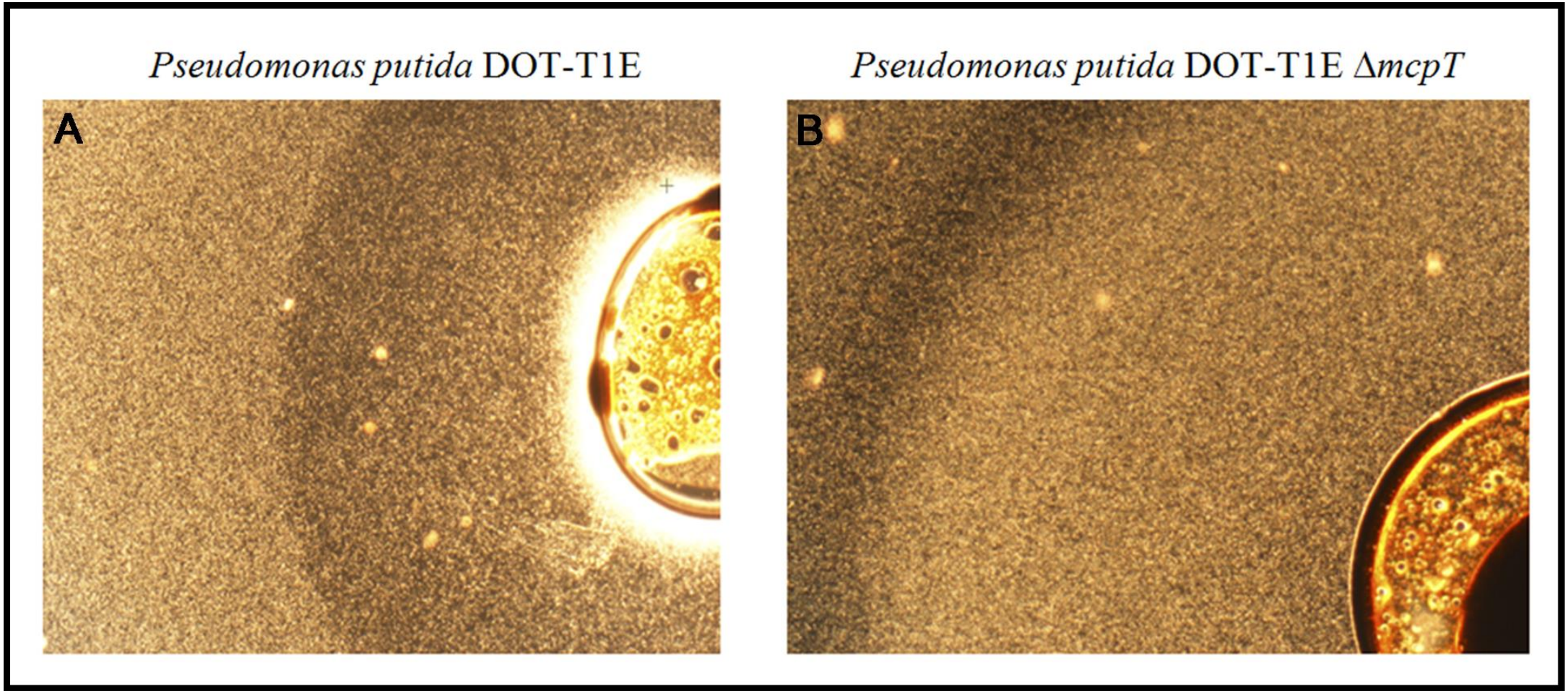

FIGURE 2. Krell et al.

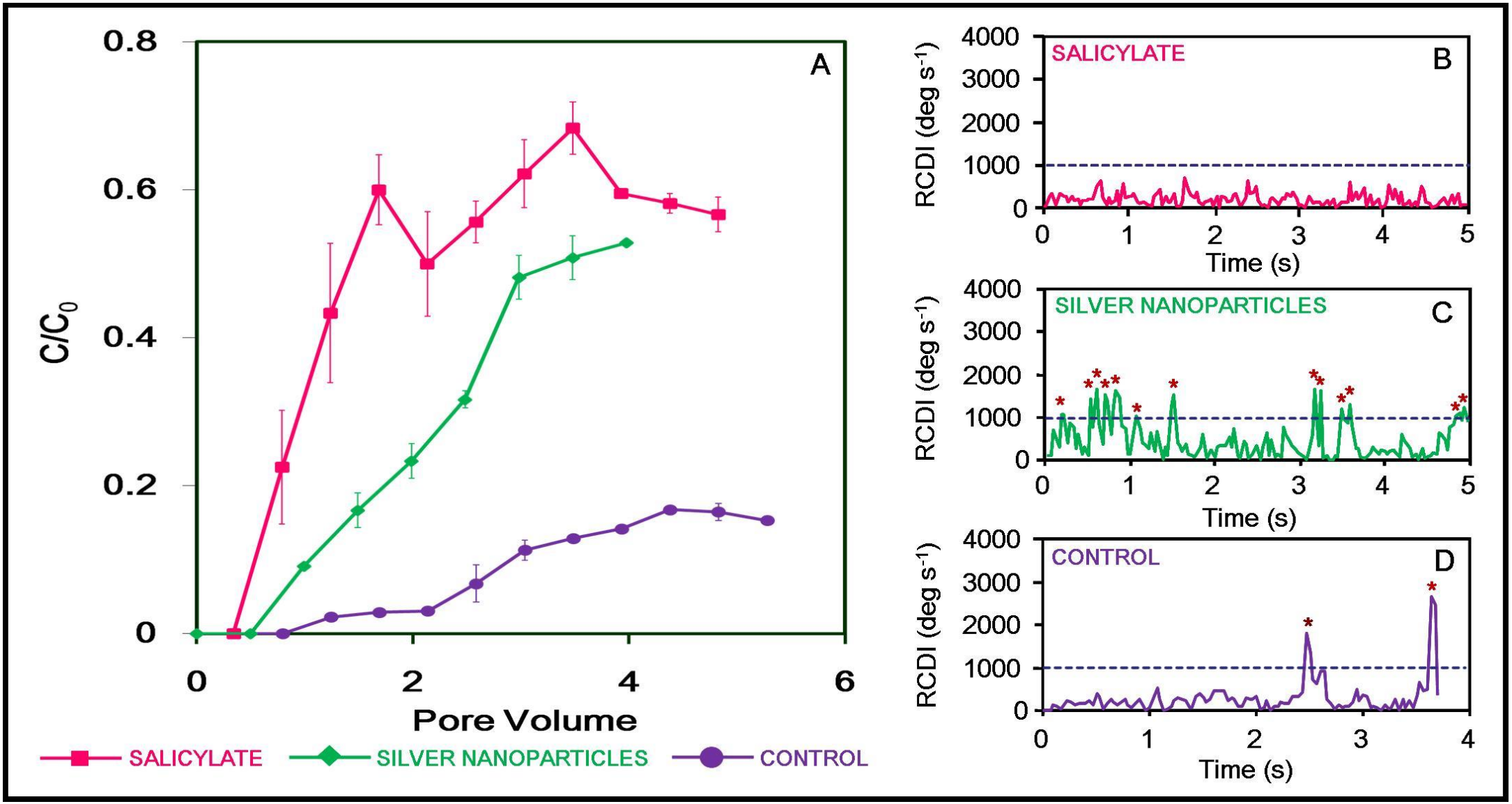

FIGURE 3. Krell et al.